PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 05 October 1999 (05.10.99)	
International application No. PCT/US98/27909	**Applicant's or agent's file reference** XY Lodo 1 PCT
International filing date (day/month/year) 31 December 1998 (31.12.98)	**Priority date** (day/month/year) 31 December 1997 (31.12.97)
Applicant SEIDEL, George, E. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

29 July 1999 (29.07.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Jean-Marie McAdams

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
XY Lodo 1 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 98/ 27909	31/12/1998	31/12/1997
Applicant		
XY, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   SEX-SPECIFIC INSEMINATION OF MAMMALS WITH LOW NUMBER OF SPERM CELLS

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/27909

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N5/06 A61D19/02 A01K67/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOHNSON L.A. ET AL: "Sex preselection in rabbits: live births fromX and Y sperm separated by DNA and cell sorting" BIOLOGY OF REPRODUCTION, vol. 41, 1989, pages 199-203, XP002103476 see page 199-200, "semen preparation and flow sorting"; see pages 201-203, "discussion" --- -/--	1-19,21, 22, 24-29, 43-50, 121-123, 125-137, 140-145, 148-177, 181,182

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 May 1999	07/06/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fernandez y Branas,F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 135 759 A (JOHNSON LAWRENCE A) 4 August 1992 cited in the application see column 4, line 44 - column 6, line 25	1-19,21, 22, 26-29, 43-50, 121-123, 125-137, 140-145, 148-177, 181,182
X	SEIDEL, G. E., JR. (1) ET AL: "Uterine horn insemination of heifers with very low numbers of nonfrozen and sexed spermatozoa." THERIOGENOLOGY, (DEC., 1997) VOL. 48, NO. 8, PP. 1255-1264. ISSN: 0093-691X., XP002103477 see the whole document	1-19,21, 22, 24-29, 43-50, 121-137, 140-145, 148-177, 181,182
X	WO 96 12171 A (UNIV WASHINGTON ;DEN ENGH GER VAN (US)) 25 April 1996 cited in the application see page 7, line 6 - line 29; figure 1	37-42, 51-53, 55-67, 78-82, 91-101, 103-120
A	DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US HAWK H W ET AL: "FERTILIZATION RATES IN SUPEROVULATING COWS AFTER DEPOSITION OF SEMEN ON THE INFUNDIBULUM NEAR THE UTEROTUBAL JUNCTION OR AFTER INSEMINATION WITH HIGH NUMBERS OF SPERM." XP002103478 see abstract & THERIOGENOLOGY, (1988) 29 (5), 1131-1142. CODEN: THGNBO. ISSN: 0093-691X.,	163-177

INTERNATIONAL SEARCH REPORT

[redacted]ation on patent family members

[redacted]rnational Application No
[redacted]T/US 98/27909

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5135759 A	04-08-1992	AT 142788 T	15-09-1996
		AU 623016 B	30-04-1992
		AU 5664290 A	29-11-1990
		DE 69028526 D	17-10-1996
		DE 69028526 T	06-02-1997
		DK 471758 T	10-03-1997
		EP 0471758 A	26-02-1992
		ES 2091823 T	16-11-1996
		HK 1000074 A	14-11-1997
		JP 2552582 B	13-11-1996
		JP 4501364 T	12-03-1992
		WO 9013303 A	15-11-1990
WO 9612171 A	25-04-1996	AU 698929 B	12-11-1998
		AU 3958295 A	06-05-1996
		CA 2201324 A	25-04-1996
		EP 0786078 A	30-07-1997
		JP 10507524 T	21-07-1998

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
XY Lodo 1 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 98/ 27909	31/12/1998	31/12/1997
International Patent Classification (IPC) or national classification and IPC		
C12N5/06		
Applicant		
XY, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 13 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consists of a total of 34 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
29/07/1999	25.04.00
Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	B. Heimann-Pohl

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US98/27909

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-31 as originally filed

Claims, No.:

1-164 with telefax of 20/04/2000

Drawings, sheets:

1/4-4/4 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☒ restricted the claims.

☒ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-164.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-140, 144, 145,148, 149, 151-164
	No:	Claims	141-143, 146, 147, 150
Inventive step (IS)	Yes:	Claims	1-140, 144, 145, 148, 149, 151-164
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-164
	No:	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations n the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1). The present application relates to a method of producing a mammal having a predetermined sex, comprising insemination of said mammal with a sorted artificial insemination sample having from about 10% to about 50% of the number of sperm cells relative to the typical artificial insemination dosage. The application further relates to improved flow cytometer systems and to a method of producing multiple, embryos having a predetermined sex from a female mammal comprising creating superovulation in said mammal.

2). The application contains 7 inventions (which were all searched) since for all of them additional fees were paid they are all subject for examination.

Invention 1:
claims 1-38, method of producing a mammal having a predetermined sex which mammal had to be inseminated with a sorted artificial insemination sample having from about 10% to about 50% of the number of sperm cells relative to the typical artificial insemination dosage and fertilization of at least one egg should be at success levels statistically comparable ($p>0.1$) to a typical unsorted artificial insemination dosage.

Invention 2:
claims 39-73, method of producing a **bovine** mammal having a predetermined sex which mammal had to be inseminated with a sorted artificial insemination sample having between no more than one hundred thousand to no more than three hundred thousand sorted bovine sperm cells, at success levels of at least 50% and where sorting involves a sheath fluid environment which contains about 2.9% sodium citrate.

Invention 3:
claims 74-104, method of producing an **equine** mammal having a predetermined sex which mammal had to be inseminated with a sorted artificial insemination sample having between no more than one million to no more than twenty five million sorted equine sperm cells, at success levels of at least 35%, 41%, 50% and at least 90% and where sorting involves a sheath fluid environment which contains hepes buffered medium.

Invention 4:
claims 105-140, an improved flow cytometer system for isolating **sperm c lls** comprising a pre-sort and a post-sort sperm cell fluid environment.

Invention 5:
claims 141-149, an improved flow cytometer system for isolating **sperm cells** comprising a collector having a cushioning element which cushioning element comprises initial collection fluid in the bottom of said collector and wherein said collector has a configuration sufficiently large to avoid impact of said sperm cells with said collector.

Invention 6:
claims 150-154, an improved flow cytometer system for isolating **sperm cells** comprising a collector configured to avoid impact between said sperm cells and said collector.

Invention 7:
claims 155-164, a method of producing multiple, embryos having a predetermined sex from a female mammal comprising creating superovulation in said mammal.

2). The following prior art cited in the international Search Report is considered to be relevant for the above claims.

D1: JOHNSON L.A. ET AL: 'Sex preselection in rabbits: live births from X and Y sperm separated by DNA and cell sorting' BIOLOGY OF REPRODUCTION, vol. 41, 1989, pages 199-203, XP002103476
D2: US-A-5 135 759 (JOHNSON LAWRENCE A) 4 August 1992 cited in the application
D3: SEIDEL, G. E., JR. (1) ET AL: 'Uterine horn insemination of heifers with very low numbers of nonfrozen and sexed spermatozoa.' THERIOGENOLOGY, (DEC., 1997) VOL. 48, NO. 8, PP. 1255-1264. ISSN: 0093-691X., XP002103477
D4: WO 96 12171 A (UNIV WASHINGTON ;DEN ENGH GER VAN (US)) 25 April 1996 cited in the application
D5: DATABASE BIOSIS BIOSCIENCES INFORMATION SERVICE,

PHILADELPHIA, PA, US HAWK H W ET AL: 'FERTILIZATION RATES IN SUPEROVULATING COWS AFTER DEPOSITION OF SEMEN ON THE INFUNDIBULUM NEAR THE UTEROTUBAL JUNCTION OR AFTER INSEMINATION WITH HIGH NUMBERS OF SPERM.' XP002103478 & THERIOGENOLOGY, (1988) 29 (5), 1131-1142. CODEN: THGNBO. ISSN: 0093-691X.,

D1 relates to a method of producing a mammal (rabbits) of predetermined sex using $3x10^5$ sperm per dose which sperm was sorted according to sex by flow cytometry (approximately 97 sorts/sec) by surgical insemination into the uterus.

D2 as D1 relates to a method of producing a mammal (rabbits) of predetermined sex using $3x10^5$ sperm per dose which sperm was sorted according to sex by flow cytometry (see col. 4 "the overall flow rate was approximately 2500 intact sperm per second. the intact X- and Y- bearing sperm fractions were sorted simultaneously from the population of input sperm at a rate of 80-90 sperm of each type per second) . D2 extends this teaching to bovine sperm (claim 4).

D2 further mentions that the method of staining, the sheath fluid, and the collecting fluid are important factors for maintaining sperm viability.
Neither D1 nor D2 mentions whether the insemination dosage used represents a "low dose" or the typical unsorted artificial insemination sample for rabbits.

D3 is published by one of the inventors of the present application. D3 discloses insemination of heifers with very low numbers of spermatozoa sorted according to their sex by flow cytometry.
Moreover the present application on page 24 lines 21-27 refers to D3 in connection with the examples of the present application: "Further a summary of some experiments is contained in the article "Uterine Horn Insemination of Heifers With Very Low Numbers of Non-frozen and Sexed Spermatozoa. This article summarizes some of the data showing the efficacy of the present invention.".

D3 (page 1258) discloses that the cells were sorted at high speed at room temperature into microfuge tubes containing 100µl of TEST-yolk (20%) extender

with or without seminal plasma.

D3 does not mention a specific sheath fluid environment during sorting, a pre-sort and a post-sort sperm cell fluid environment or a collector/ collection tube which is at least 15 mm wide.

Moreover, on page 1262, third paragraph relating to unsorted sperm, D3 mentions that "we clearly demonstrated that pregnancy rates of 40-50% are attainable in Holstein heifers using 1 to 2.5 x 10^5 total sperm cells per inseminate. Pregnancy rates with these low sperm numbers were clearly below those of the controls (2.5 x 10^6 sperm/dose), so these very low doses will usually be inappropriate for commercial purposes."

D4 relates to a high speed flow cytometer droplet formation system.

D5 relates to superovulation in dairy cows which were treated with follicle-stimulating hormone and with prostaglandin $F2_{\alpha}$ to regulate the time of estrus. D5 did not use sperm cells sorted according to sex but unsorted sperm cells for artificial insemination.

4). Invention 1:

4.1). Novelty

The method of claims 1-38 relating to producing a mammal having a predetermined sex which mammal had to be inseminated with a sorted artificial insemination sample having from about 10% to about 50% of the number of sperm cells relative to the typical artificial insemination dosage and fertilization of at least one egg should be at success levels statistically comparable ($p>0.1$) to a typical unsorted artificial insemination dosage appears to be novel with regard to D3.
D3 is apparently not enabling for the "at least about 38 micro-molar content of stain", "the chemically coordinated sheath fluid....", the species/buffer combination , the citrate collection fluid containing about 6% egg yolk and high speed sorting , the use of FSH, the step of injecting a dosage of follicle stimulating hormone a

plurality of times a day and the flow cytometer system having the above mentioned characteristics (sheath fluid, etc.).

Although D2 mentions in claim 4 bovine sperm this document is not considered as enabling for an insemination sample having a low number of said sperm cells relative to the typical artificial insemination sample.

4.2). Inventive Step

The problem to be solved by invention 1 appears to be the provision undamaged or less damaged sorted sperm cells which allows establishing sorted artificial insemination samples having from about 10% to 50% of the number of sperm cells relative to the typical artificial insemination dosage, still allowing fertilizing eggs at success levels statistically comparable ($p>0.1$) to the typical unsorted artificial insemination dosage for commercial purposes.
With regard to D3, page 1262, third paragraph relating to unsorted sperm, ("we clearly demonstrated that pregnancy rates of 40-50% are attainable in Holstein heifers using 1 to 2.5 x 10^5 total sperm cells per inseminate. Pregnancy rates with these low sperm numbers were clearly below those of the controls (2.5 x 10^6 sperm/dose), so these very low doses will usually be inappropriate for commercial purposes.") the skilled person would have had no incentive to try to establish sorted artificial insemination samples at these low numbers of sperm, since even unsorted sperm at these low numbers were considered to be inappropriate.

The above problem of the present application is solved by a combination of features not obviously derivable from D3. Therefore an inventive step can be acknowledged for claims 1-38.

5). Invention 2

5.1). Novelty and Inventive Step

The subject matter of invention 2, claims 39-73 appears to be novel and not obviously derivable from the cited prior art.

The method of producing a **bovine** mammal having a predetermined sex which mammal had to be inseminated with a sorted artificial insemination sample having between no more than one hundred thousand to no more than three hundred thousand sorted bovine sperm cells, at success levels of at least 50% and where sorting involves a sheath fluid environment which contains about 2.9% sodium citrate solves the problem of providing undamaged or less damaged sorted sperm cells which allows establishing insemination samples having a low number of sperm cell relative to the typical artificial insemination dosage, still allowing fertilizing eggs at success levels statistically comparable to the typical unsorted artificial insemination dosage (see also arguments under 4.2). for invention 1 with regard to D3).

6). Invention 3

6.1). Novelty and Inventive Step

The subject matter of invention 3, claims 74-104, appears to be novel and inventive with regard to the cited prior art.
The method of producing an **equine** mammal having a predetermined sex which mammal had to be inseminated with a sorted artificial insemination sample having between no more than one million to no more than twenty five million sorted equine sperm cells, at success levels of at least 35%, 41%, 50% and at least 90% and where sorting involves a sheath fluid environment which contains hepes buffered medium solves the problem of providing undamaged or less damaged sexed sperm cells which allows establishing insemination samples having a low number of sperm cell relative to the typical artificial insemination dosage, still allowing fertilizing eggs at success levels statistically comparable to the typical unsexed artificial insemination dosage (see also arguments under 4.2). for invention 1 with regard to D3).

7). Invention 4

7.1). Novelty

None of the cited documents apparently discusses a chemically coordinated

sheath fluid source which creates a sheath fluid environment for sperm cells which is selected to be coordinated with both a pre-sort and post- sort sperm cell fluid environment in order to impose less stress to the sperm cells to be sorted. D2, although mentioning the problem that sheath fluid is one of the factors involved in maintaining sperm viability, does not refer to a chemically coordinated sheath fluid which creates both a pre-sort and post-sort sperm cell fluid environment. Thus the subject matter of claims 105-140 appears to be novel.

7.2). Inventive Step

The problem underlying invention 4 is already known from D2 (sheath fluid is one of the factors involved in maintaining sperm viability).
The alleged solution to said problem is a chemically coordinated sheath fluid source which creates a sheath fluid environment for sperm cells which is selected to be coordinated with both a pre-sort and post- sort sperm cell fluid environment which, however, does not appear to be obviously derivable from D2.
The subject matter of claims 105-140 appears to involve an inventive step.

8). Invention 5

8.1). Novelty and Inventive Step

D3 (page 1258) discloses that the cells were sorted at high speed at room temperature into microfuge tubes containing 100µl of TEST-yolk (20%) extender with or without seminal plasma. By the unclear wording "a collector having a cushioning element, wherein said cushioning element comprises initial collection fluid in the bottom of the said collector and wherein said collector has a configuration sufficiently large to avoid impact of said sperm cells with said collector" (step g. in claim 141) it appears that the extender and the microfuge tube of D3 is not excluded. Thus the subject matter of claims 141-143, 146 and 147 seems to lack novelty.

The subject matter claims 144, 145, 148 and 149 appears to be novel and inventive with regard to D3, because the specific features in these claims are not

specifically mentioned or obviously derivable from said document.

9). Invention 6

9.1). Novelty

By the wording "collector configured to avoid impact between said sperm cells and said collector" the tube used in D3 does not appear to be excluded therefore claim 150 lacks novelty.

The subject matter claims 151-154 appears to be novel and inventive with regard to D3, because the specific features in these claims are not specifically mentioned or obviously derivable from said document.

10). Invention 7

10.1).Novelty and Inventive Step

The subject matter of invention 7, claims 155-164, appears to be novel with regard to D3 and also not obviously derivable by the combination of D3 with D5.

11). Clarity (Art.6 PCT)

The examination of the claims with regard to clarity is performed for inventions 1-7 without separating these inventions.

11.1).From the document Reproduction In Domestic Animals, edited by H.H.Cole and P.T.Cupps, Third Edition 1977, Chapter entiteled "Artificial Insemination" pages 264, 265, and 278, specifically Table II, and pages 565-572, specifically page 569 first paragraph and Figure 18-6, it appears that the skilled person would know what the terms "typical unsorted artificial insemination sample/dosage" (in e.g. claim 1) and "generally regarded as optimal" (in claims 10, 49, 85) mean in the art.

11.2). The wording "a collector having a cushioning element, wherein said cushioning element comprises initial collection fluid in the bottom of the said collector and wherein said collector has a configuration sufficiently large to avoid impact of said sperm cells with said collector" and "collector configured to avoid impact between said sperm cells and said collector" lacks clarity because "initial collection fluid" does not specify the fluid , "sufficiently large" is a relative term and the collector is only defined through the result to be achieved by its configuration (to avoid impact) (PCT International Preliminary Examination Guidelines, C III-4).

REPLACED BY ARTICLE 34

VI. CLAIMS

What is claimed is:

1. A method of producing a mammal having a predetermined sex comprising the steps of:
   a. collecting sperm cells from a male species of a mammal;
   b. determining the sex characteristic of a plurality of said sperm cells;
   c. sorting said sperm cells according to the determination of their sex characteristic;
   d. establishing an insemination sample having a low number of said sperm cells relative to the typical artificial insemination dosage;
   e. inserting at least a portion of said insemination sample into a female species of said mammal;
   f. fertilizing at least one egg within said female species of said mammal at success levels statistically comparable to the typical unsexed artificial insemination dosage; and
   g. producing an offspring mammal of the desired sex.

2. A method of producing a mammal having a predetermined sex as described in claim 1 wherein said step of fertilizing at least one egg within said female species of said mammal at success levels statistically comparable to the typical artificial insemination dosage comprises the step of fertilizing at least one egg within said female species of said mammal at success levels selected from the group consisting of at least 35%, at least 41%, at least 50%, and at least 90%.

3. A method of producing a mammal having a predetermined sex as described in claim 2 wherein said step of collecting sperm cells from a male species of a mammal comprises the step of collecting sperm cells from a male species of a mammal selected from the group consisting of bovines and equines.

4. A method of producing a mammal having a predetermined sex as described in claim 3 wherein said step of establishing an insemination sample having a low number of said

sperm cells relative to the typical artificial insemination dosage comprises the step of establishing an insemination sample having no more than ten percent of the typical number of sperm provided in a typical, unsexed artificial insemination event.

5. A method of producing a mammal having a predetermined sex as described in claim 3 wherein said step of establishing an insemination sample having a low number of said sperm cells relative to the typical artificial insemination dosage comprises the step of establishing an insemination sample selected from the group consisting of: a bovine insemination sample of no more than one hundred thousand sperm cells, a bovine insemination sample of no more than two hundred fifty thousand sperm cells, a bovine insemination sample of no more than three hundred thousand sperm cells, an equine insemination sample of no more than one million sperm cells, an equine insemination sample of no more than five million sperm cells, an equine insemination sample of no more than ten million sperm cells, and an equine insemination sample of no more than twenty-five million sperm cells.

6. A method of producing a mammal having a predetermined sex as described in claim 1, 2, 3, 4, or 5 wherein said steps of inserting at least a portion of said insemination sample into a female species of said mammal and fertilizing at least one egg within said female species of said mammal at success levels statistically comparable to the typical unsexed artificial insemination dosage are each accomplished in a field environment.

7. A method of producing a mammal having a predetermined sex as described in claim 6 wherein said steps of inserting at least a portion of said insemination sample into a female species of said mammal and fertilizing at least one egg within said female species of said mammal at success levels statistically comparable to the typical unsexed artificial insemination dosage in a field environment comprises the steps of repetitively inserting a significant number of insemination samples into a significant number of female specie of said mammal in rapid succession and in farm or ranch conditions.

8. A method of producing a mammal having a predetermined sex as described in claim 1, 2, 3, 4, or 5 wherein said mammal has uterine horns and wherein said step of inserting at least a portion of said insemination sample into a female species of said mammal comprises the step of inserting said insemination sample both ipsi- and contra-lateral within the uterine horns of said mammal.

9. A method of producing a mammal having a predetermined sex as described in claim 1, 2, 3, 4, or 5 wherein said mammal has at least one uterine horn and wherein said step of inserting at least a portion of said insemination sample into a female species of said mammal comprises the step of inserting said insemination sample deep within said uterine horn.

10. A method of producing a mammal having a predetermined sex as described in claim 8 wherein said step of inserting at least a portion of said insemination sample into a female species of said mammal further comprises the step of inserting said insemination sample deep within said uterine horns.

11. A method of producing a mammal having a predetermined sex as described in claim 9 wherein step of inserting at least a portion of said insemination sample into a female species of said mammal further comprises the step of inserting said insemination sample within said uterine horn through the use of embryo transfer equipment.

12. A method of producing a mammal having a predetermined sex as described in claim 10 wherein step of inserting at least a portion of said insemination sample into a female species of said mammal further comprises the step of inserting said insemination sample within said uterine horn through the use of embryo transfer equipment.

13. A method of producing a mammal having a predetermined sex as described in claim 8 wherein said step of inserting at least a portion of said insemination sample into a female species of said mammal comprises the step of inserting said insemination sample twelve

hours after the time which is generally regarded as optimal for a single artificial insemination.

14. A method of producing a mammal having a predetermined sex as described in claim 12 wherein said step of establishing an insemination sample having a low number of said sperm cells relative to the typical artificial insemination dosage comprises the step of establishing an unfrozen insemination sample, wherein said step of sorting said sperm cells according to the determination of their sex characteristic occurs at a sorting time, and wherein said step of inserting at least a portion of said insemination sample into a female species of said mammal occurs not later than about seventeen hours from said sorting time.

15. A method of producing a mammal having a predetermined sex as described in claim 12 wherein said step of establishing an insemination sample having a low number of said sperm cells relative to the typical artificial insemination dosage comprises the step of establishing an unfrozen insemination sample, wherein said step of sorting said sperm cells according to the determination of their sex characteristic occurs at a sorting time, and wherein said step of inserting at least a portion of said insemination sample into a female species of said mammal occurs not later than about ten hours from said sorting time.

16. A method of producing a mammal having a predetermined sex as described in claim 6 wherein said step of determining the sex characteristic of a plurality of said sperm cells comprises the step of staining said cells with a high concentration of stain.

17. A method of producing a mammal having a predetermined sex as described in claim 6 wherein said step of determining the sex characteristic of a plurality of said sperm cells comprises the step of staining said cells with at least about 38 micro-molar content of stain.

18. A method of producing a mammal having a predetermined sex as described in claim 1 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic comprise the steps of:

a. establishing a cell source which supplies cells to be sorted;

b. chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment;

c. sensing a property of said cells;

d. discriminating between cells having a desired sex characteristic; and

e. collecting cells having the desired sex characteristic.

19. A method of producing a mammal having a predetermined sex as described in claim 6 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic comprise the steps of:

a. establishing a cell source which supplies cells to be sorted;

b. chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment;

c. sensing a property of said cells;

d. discriminating between cells having a desired sex characteristic; and

e. collecting cells having the desired sex characteristic.

20. A method of producing a mammal having a predetermined sex as described in claim 1 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic comprise the steps of:

a. establishing a cell source which supplies bovine sperm cells to be sorted;

b. establishing a sheath fluid for said bovine sperm cells which contains about 2.9% sodium citrate;

c. sensing a property of said cells;

d. discriminating between cells having a desired sex characteristic; and

e. collecting cells having the desired sex characteristic.

21. A method of producing a mammal having a predetermined sex as described in claim 1 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic comprise the steps of:

a. establishing a cell source which supplies equine sperm cells to be sorted;

b. establishing a sheath fluid for said equine sperm cells which contains a hepes buffered medium;

c. sensing a property of said cells;

d. discriminating between cells having a desired sex characteristic; and

e. collecting cells having the desired sex characteristic.

22. A method of producing a mammal having a predetermined sex as described in claim 1 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic comprise the steps of:

a. establishing a cell source which supplies cells to be sorted;

b. establishing a sheath fluid for said cells;

c. sensing a property of said cells;

d. discriminating between cells having a desired sex characteristic; and

e. collecting cells having the desired sex characteristic while cushioning said cells from impact with a collection container.

23. A method of producing a mammal having a predetermined sex as described in claim 1 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic comprise the steps of:

a. establishing a cell source which supplies bovine sperm cells to be sorted;

b. establishing a sheath fluid for said bovine sperm cells;

c. sensing a property of said bovine sperm cells;

d. discriminating between bovine sperm cells having a desired sex characteristic; and

e. collecting bovine sperm cells having the desired sex characteristic in a citrate collection fluid containing about six percent egg yolk prior to commencing said step of collecting.

24. A method of producing a mammal of a desired sex comprising the step of producing said mammal using the processes according to any of claims 18, 19, 20, 21, 22, or 23.

25. A method of producing a mammal of a desired sex as described in claim 24 and further comprising the step of sorting said cells at a high speed.

26. A method of producing a mammal having a predetermined sex as described in claim 1 and further comprising the step of using an ovulatory pharmaceutical to cause multiple eggs to be produced and wherein said step of fertilizing at least one egg within said female species of said mammal at success levels statistically comparable to the typical unsexed artificial insemination dosage comprises the step of fertilizing a plurality of said eggs to produce multiple, sexed embryos.

27. A method of producing a mammal having a predetermined sex as described in claim 26 wherein said step of using an ovulatory pharmaceutical to cause multiple eggs to be produced comprises the step of injecting a dosage of follicle stimulating hormone a plurality of times a day.

28. A method of producing a mammal having a predetermined sex as described in claim 27 wherein said step of injecting said dosage of follicle stimulating hormone a plurality of times a day comprises the step of injecting said follicle stimulating hormone in approximately half day increments at a dosage level of 6, 6, 4, 4, 2, 2, 2, and 2 mg between days 9 and 12 inclusive of the estrus cycle and further comprising the step of

injecting 25 and 12.5 mg of prostaglandin F-2-alpha on the sixth and seventh dosages, respectively, of said follicle stimulating hormone.

29. A method of producing a mammal having a predetermined sex as described in claim 26 and further comprising the steps of:

a. staining sperm cells of a male mammal;

b. sorting according to said sex of said sperm cells through the use of high speed flow cytometry; and

c. concentrating said sorted sperm cells.

30. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies cells to be analyzed by the flow cytometer;

b. a sheath fluid source which creates a sheath fluid environment for said cells which contains about 2.9% sodium citrate;

c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;

e. a cell sensing system which responds to said cells;

f. a sorter discrimination system which acts to sort cells having a desired characteristic; and

g. a collector into which cells having a desired characteristic are placed.

31. An improved flow cytometer system for isolating desired cells as described in claim 30 wherein said cell source comprises bovine sperm cells.

32. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies cells to be analyzed by the flow cytometer;

b. a sheath fluid source which creates a sheath fluid environment for said cells which contains a hepes buffered medium;

c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;

e. a cell sensing system which responds to said cells;

f. a sorter discrimination system which acts to sort cells having a desired characteristic; and

g. a collector into which cells having a desired characteristic are placed.

33. An improved flow cytometer system for isolating desired cells as described in claim 32 wherein said cell source comprises equine sperm cells.

34. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies cells to be analyzed by the flow cytometer;

b. a sheath fluid source which creates a sheath fluid environment for said cells;

c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;

e. a cell sensing system which responds to said cells;

f. a sorter discrimination system which acts to sort cells having a desired characteristic; and

g. a collector into which cells having a desired characteristic are placed and which comprises a citrate collector fluid containing about six percent egg yolk.

35. An improved flow cytometer system for isolating desired cells as described in claim 34 wherein said sheath fluid source comprises a solution containing about 2.9% sodium citrate.

36. An improved flow cytometer system for isolating desired cells as described in claim 34 or 35 wherein said cell source comprises bovine sperm cells.

37. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies cells to be analyzed by the flow cytometer;

b. a sheath fluid source which creates a sheath fluid environment for said cells;

c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;

e. a cell sensing system which responds to said cells;

f. a sorter discrimination system which acts to sort cells having a desired characteristic; and

g. a collector into which cells having a desired characteristic are placed and which comprises a test tube having the physical characteristics of a stream-matched container.

38. An improved flow cytometer system for isolating desired cells as described in claim 37 wherein said cell source comprises cells which are mechanically delicate.

39. An improved flow cytometer system for isolating desired cells as described in claim 30, 32, or 34 wherein said cell source comprises cells which are hyper-responsive to a chemical composition in a surrounding fluid environment.

40. An improved flow cytometer system for isolating desired cells as described in claim 30, 32, 34, or 37 wherein said collector is used to provide a low dose of sperm.

41. An improved flow cytometer system for isolating desired cells as described in claim 40 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are

part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.

42. An improved flow cytometer system for isolating desired cells as described in claim 37 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.

43. A sexed sperm specimen produced according to a system as described in any of claims 30, 32, 34, 35, 37, 51, 59, 63, 105, 107, 88, or 101.

44. A sexed sperm specimen as described in claim 43 wherein said collector is used to provide a low dose of sperm.

45. A sexed sperm specimen as described in claim 43 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.

46. A sexed sperm specimen as described in claim 44 wherein said collector is used to provide a low dose of sperm.

47. A mammal produced through use of a sexed sperm specimen produced according to a system as described in any of claims 30, 32, 34, 35, 37, 51, 59, 63, 105, 107, 88, or 101

48. A mammal as described in claim 47 wherein said mammal is produced through use of a low dose of sperm.

49. A mammal as described in claim 47 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.

50. A mammal as described in claim 48 wherein said mammal is produced through use of a low dose of sperm.

51. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies cells to be analyzed by the flow cytometer;

b. a chemically coordinated sheath fluid source which creates a sheath fluid environment for said cells which is selected to be coordinated with both a pre-sort and a post-sort cell fluid environment;

c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;

e. a cell sensing system which responds to said cells;

f. a sorter discrimination system which acts to sort cells having a desired characteristic; and

g. a collector into which cells having a desired characteristic are placed.

52. An improved flow cytometer system for isolating desired cells as described in claim 51 wherein said pre-sort and post-sort cell fluid environments contain at least one hyper-responsive chemical composition to which said cells are particularly responsive and wherein said chemically coordinated sheath fluid source minimizes changes to said hyper-responsive chemical composition.

53. An improved flow cytometer system for isolating desired cells as described in claim 52 wherein said hyper-responsive chemical composition comprises a metabolic chemical composition.

54. An improved flow cytometer system for isolating desired cells as described in claim 52 wherein said hyper-responsive chemical composition comprises a citrate.

55. An improved flow cytometer system for isolating desired cells as described in claim 51 wherein said cell source creates said pre-sort cell fluid environment and wherein said collector creates said post-sort cell fluid environment.

56. An improved flow cytometer system for isolating desired cells as described in claim 52 wherein said cell source comprises non-repairing cells.

57. An improved flow cytometer system for isolating desired cells as described in claim 56 wherein said cell source comprises cells which have non-transcribing DNA.

58. An improved flow cytometer system for isolating desired cells as described in claim 56 wherein said cell source comprises cells which have non-replicating DNA.

59. An improved flow cytometer system for isolating desired cells as described in claim 56 wherein said cell source comprises sperm cells.

60. An improved flow cytometer system for isolating desired cells as described in claim 52 or 54 wherein said cell source comprises bovine sperm cells.

61. An improved flow cytometer system for isolating desired cells as described in claim 52 wherein said cell source comprises equine sperm cells.

62. An improved flow cytometer system for isolating desired cells as described in claim 51 wherein said cell source comprises cells which are hyper-responsive to a chemical composition in a sheath fluid environment.

63. An improved flow cytometer system for isolating desired cells as described in claim 59 wherein said collector is used to provide a low dose of sperm.

64. An improved flow cytometer system for isolating desired cells as described in claim 63 wherein said low dose of sperm comprises a dosage of less than about ten percent of said typical dosage.

65. An improved flow cytometer system for isolating desired cells as described in claim 63 wherein said sperm cells comprise bovine sperm cells and wherein said low dose of sperm comprises a dosage of less than about five hundred thousand sperm.

66. An improved flow cytometer system for isolating desired cells as described in claim 63 wherein said sperm cells comprise bovine sperm cells and wherein said low dose of sperm comprises a dosage of less than about three hundred thousand sperm.

67. An improved flow cytometer system for isolating desired cells as described in claim 63 wherein said sperm cells comprise equine sperm cells and wherein said low dose of sperm comprises a dosage of less than about ten million sperm.

68. An improved flow cytometer system for isolating desired cells as described in claim 54 wherein said chemically coordinated sheath fluid source comprises a solution containing about 2.9% sodium citrate.

69. An improved flow cytometer system for isolating desired cells as described in claim 68 wherein said cell source comprises bovine sperm cells.

70. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies cells to be analyzed by the flow cytometer;

b. a sheath fluid source which creates a sheath fluid environment for said cells which comprises a solution containing about 2.9% sodium citrate;

c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;

e. a cell sensing system which responds to said cells;

f. a sorter discrimination system which acts to sort cells having a desired characteristic; and

g. a collector into which cells having a desired characteristic are placed.

71. An improved flow cytometer system for isolating desired cells as described in claim 69 wherein said collector is used to provide sperm for artificial insemination.

72. An improved flow cytometer system for isolating desired cells as described in claim 69 wherein said collector is used to provide a low dose of sperm for artificial insemination.

73. An improved flow cytometer system for isolating desired cells as described in claim 54 wherein said chemically coordinated sheath fluid source comprises a solution containing a hepes buffered medium.

74. An improved flow cytometer system for isolating desired cells as described in claim 73 wherein said cell source comprises equine sperm cells.

75. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies cells to be analyzed by the flow cytometer;

b. a sheath fluid source which creates a sheath fluid environment for said cells which comprises a solution containing a hepes buffered medium;

c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;

e. a cell sensing system which responds to said cells;

f. a sorter discrimination system which acts to sort cells having a desired characteristic; and

g. a collector into which cells having a desired characteristic are placed.

76. An improved flow cytometer system for isolating desired cells as described in claim 74 wherein said collector is used to provide sperm for artificial insemination.

77. An improved flow cytometer system for isolating desired cells as described in claim 74 wherein said collector is used to provide a low dose of sperm for artificial insemination.

78. An improved flow cytometer system for isolating desired cells as described in claim 51, 52, 54, 59, 72, or 77 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.

79. An improved flow cytometer system for isolating desired cells as described in claim 78 wherein said high speed cell sorter sorts said cells to be analyzed at a rate of at least about five hundred sorts per second.

80. An improved flow cytometer system for isolating desired cells as described in claim 78 wherein said high speed cell sorter operates at a pressure of at least about fifty pounds per square inch.

81. An improved flow cytometer system for isolating desired cells as described in claim 59 wherein said collector comprises a container comprising a cushioning element.

82. An improved flow cytometer system for isolating desired cells as described in claim 81 wherein said container comprises a wide collection tube.

83. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies bovine sperm cells to be analyzed by the flow cytometer;

b. a chemically coordinated sheath fluid source which creates a sheath fluid environment for said cells which is contains about 2.9% sodium citrate;

c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;

e. a cell sensing system which responds to said cells;

f. a sorter discrimination system which acts to sort cells having a desired characteristic;

g. a collector into which cells having a desired characteristic which contains a citrate collection fluid comprising about six percent egg yolk and which is used to provide a dosage of less than about five hundred thousand sperm;

and wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system which sorts said cells to be analyzed at a rate of at least about five hundred sorts per second and operates at a pressure of at least about fifty pounds per square inch.

84. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies equine sperm cells to be analyzed by the flow cytometer;

b. a chemically coordinated sheath fluid source which creates a sheath fluid environment for said cells which is contains a hepes buffered medium;

c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;

e. a cell sensing system which responds to said cells;

f. a sorter discrimination system which acts to sort cells having a desired characteristic;

g. a collector into which cells having a desired characteristic which contains a collection fluid comprising a hepes buffered medium and which is used to provide a dosage of less than about ten million sperm;

and wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system which sorts said cells to be analyzed at a rate of at least about five hundred sorts per second and operates at a pressure of at least about fifty pounds per square inch.

85. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies cells to be analyzed by the flow cytometer;

b. a means for minimizing the changes between a sheath fluid environment for said cells and both a pre-sort and a post-sort cell fluid environment;

c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;

e. a cell sensing system which responds to said cells;

f. a sorter discrimination system which acts to sort cells having a desired characteristic; and

g. a collector into which cells having a desired characteristic are placed.

86. An improved flow cytometer system for isolating desired cells as described in claim 85 wherein said means for minimizing the changes between a sheath fluid environment for said cells and both a pre-sort and a post-sort cell fluid environment comprises said sheath fluid.

87. An improved flow cytometer system for isolating desired cells as described in claim 85 or 86 wherein said collector has a collector fluid and wherein said means for minimizing the changes between a sheath fluid environment for said cells and both a pre-sort and a post-sort cell fluid environment comprises said collector fluid.

88. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies cells to be analyzed by the flow cytometer;

b. a sheath fluid source which creates a sheath fluid environment for said cells;

c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;

e. a cell sensing system which responds to said cells;

f. a sorter discrimination system which acts to sort cells having a desired characteristic; and

g. a collector into which cells having a desired characteristic are placed which comprises a chemically coordinated collector fluid sheath fluid source which creates a collector fluid environment for said cells which is selected to be coordinated with a prior cell fluid environment.

89. An improved flow cytometer system for isolating desired cells as described in claim 88 wherein said collector fluid comprises a nutrient which is coordinated to balance the level of said nutrient after completion of the sorting of said cells.

90. An improved flow cytometer system for isolating desired cells as described in claim 89 wherein said collector fluid comprises a citrate solution containing about six percent egg yolk.

91. An improved flow cytometer system for isolating desired cells as described in claim 89 or 90 wherein said cell source comprises cells which are hyper-responsive to a chemical composition in said collector fluid environment.

92. An improved flow cytometer system for isolating desired cells as described in claim 88 or 89 wherein said cell source comprises sperm cells.

93. An improved flow cytometer system for isolating desired cells as described in claim 90 wherein said cell source comprises bovine sperm cells.

94. An improved flow cytometer system for isolating desired cells as described in claim 88 wherein said collector is used to provide a low dose of sperm.

95. An improved flow cytometer system for isolating desired cells as described in claim 94 wherein said low dose of sperm comprises a dosage of less than about ten percent of said typical dosage.

96. An improved flow cytometer system for isolating desired cells as described in claim 94 wherein said sperm cells comprise bovine sperm cells and wherein said low dose of sperm comprises a dosage of less than about five hundred thousand sperm.

97. An improved flow cytometer system for isolating desired cells as described in claim 94 wherein said sperm cells comprise bovine sperm cells and wherein said low dose of sperm comprises a dosage of less than about three hundred thousand sperm.

98. An improved flow cytometer system for isolating desired cells as described in claim 88, 93, or 94 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.

99. An improved flow cytometer system for isolating desired cells as described in claim 98 wherein said high speed cell sorter sorts said cells to be analyzed at a rate of at least about five hundred sorts per second.

100. An improved flow cytometer system for isolating desired cells as described in claim 98 wherein said high speed cell sorter operates at a pressure of at least about fifty pounds per square inch.

101. An improved flow cytometer system for isolating desired cells as described in claim 88 wherein said sheath source comprises a chemically coordinated sheath fluid source which

creates a sheath fluid environment for said cells which is selected to be coordinated with both a pre-sort and a post-sort cell fluid environment.

102. An improved flow cytometer system for isolating desired cells as described in claim 101 wherein said chemically coordinated sheath fluid source comprises a solution containing about 2.9% sodium citrate.

103. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies cells to be analyzed by the flow cytometer;

b. a sheath fluid source which creates a sheath fluid environment for said cells;

c. a nozzle through which said cells pass while subjected to said sheath fluid environment;

d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;

e. a cell sensing system which responds to said cells;

f. a sorter discrimination system which acts to sort cells having a desired characteristic; and

g. a collector which comprises a cushioning element.

104. An improved flow cytometer system for isolating desired cells as described in claim 103 wherein said collector comprises a container comprising said cushioning element.

105. An improved flow cytometer system for isolating desired cells as described in claim 104 wherein said container comprises a wide collection tube.

106. An improved flow cytometer system for isolating desired cells as described in claim 105 wherein said wide collection tube is at least about fifteen millimeters wide.

107. An improved flow cytometer system for isolating desired cells as described in claim 104 wherein said container comprises a test tube having the physical characteristics of a stream-matched container.

108. An improved flow cytometer system for isolating desired cells as described in claim 104 wherein said cell source comprises cells which are mechanically delicate.

109. An improved flow cytometer system for isolating desired cells as described in claim 104 wherein said cell source comprises sperm cells.

110. An improved flow cytometer system for isolating desired cells as described in claim 88, 90, or 93 wherein said collector comprises a cushioning element.

111. An improved flow cytometer system for isolating desired cells as described in claim 110 wherein said collector comprises a container comprising said cushioning element.

112. An improved flow cytometer system for isolating desired cells as described in claim 111 wherein said container comprises a wide collection tube.

113. An improved flow cytometer system for isolating desired cells as described in claim 112 wherein said wide collection tube is at least about fifteen millimeters wide.

114. An improved flow cytometer system for isolating desired cells as described in claim 88 wherein said container comprises a test tube having the physical characteristics of a stream-matched container.

115. An improved flow cytometer system for isolating desired cells as described in claim 112 wherein said cell source comprises cells which are mechanically delicate.

116. An improved flow cytometer system for isolating desired cells as described in claim 115 wherein said cell source comprises sperm cells.

117. An improved flow cytometer system for isolating desired cells as described in claim 108 wherein said nozzle, oscillator, cell sensing system, and sorter discrimination system are part of a flow cytometer system and wherein said flow cytometer system comprises a high speed cell sorter.

118. An improved flow cytometer system for isolating desired cells as described in claim 117 wherein said high speed cell sorter sorts said cells to be analyzed at a rate of at least about five hundred sorts per second.

119. An improved flow cytometer system for isolating desired cells as described in claim 117 wherein said high speed cell sorter operates at a pressure of at least about fifty pounds per square inch.

120. An improved flow cytometer system for isolating desired cells comprising:

a. a cell source which supplies cells to be analyzed by the flow cytometer;
b. a sheath fluid source which creates a sheath fluid environment for said cells;
c. a nozzle through which said cells pass while subjected to said sheath fluid environment;
d. an oscillator which acts upon said sheath fluid as it passes through said nozzle;
e. a cell sensing system which responds to said cells;
f. a sorter discrimination system which acts to sort cells having a desired characteristic; and
g. a collector which comprises a means for avoiding impact between said cells and said collector.

121. A method of producing a mammal having a predetermined sex comprising the steps of:

a. collecting sperm cells from a male species of a mammal;
b. determining the sex characteristic of a plurality of said sperm cells;
c. sorting said sperm cells according to the determination of their sex characteristic;

d. establishing an insemination sample having a low number of said sperm cells relative to the typical artificial insemination dosage;

e. inserting at least a portion of said insemination sample into a female species of said mammal;

f. fertilizing at least one egg within said female species of said mammal; and

g. producing an offspring mammal of the desired sex.

122. A method of producing a mammal having a predetermined sex as described in claim 121 wherein said step of collecting sperm cells from a male species of a mammal comprises the step of collecting sperm cells from a male species of a mammal selected from the group consisting of bovines and equines.

123. A method of producing a mammal having a predetermined sex as described in claim 121 wherein said step of establishing an insemination sample having a low number of said sperm cells relative to the typical artificial insemination dosage comprises the step of establishing an insemination sample having no more than ten percent of the typical number of sperm provided in a typical, unsexed artificial insemination event.

124. A method of producing a mammal having a predetermined sex as described in claim 121 wherein said step of establishing an insemination sample having a low number of said sperm cells relative to the typical artificial insemination dosage comprises the step of establishing an insemination sample selected from the group consisting of: a bovine insemination sample of no more than one hundred thousand sperm cells, a bovine insemination sample of no more than two hundred fifty thousand sperm cells, a bovine insemination sample of no more than three hundred thousand sperm cells, an equine insemination sample of no more than one million sperm cells, an equine insemination sample of no more than five million sperm cells, an equine insemination sample of no more than ten million sperm cells, and an equine insemination sample of no more than twenty-five million sperm cells.

125. A method of producing a mammal having a predetermined sex as described in claim 121 wherein said steps of inserting at least a portion of said insemination sample into a female species of said mammal and fertilizing at least one egg within said female species of said mammal are each accomplished in a field environment.

126. A method of producing a mammal having a predetermined sex as described in claim 121 wherein step of inserting at least a portion of said insemination sample into a female species of said mammal further comprises the step of inserting said insemination sample within said uterine horn through the use of embryo transfer equipment.

127. A method of producing a mammal having a predetermined sex as described in claim 121 wherein said step of determining the sex characteristic of a plurality of said sperm cells comprises the step of staining said cells with a high concentration of stain.

128. A method of producing a mammal having a predetermined sex as described in claim 121 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic comprise the steps of:

a. establishing a cell source which supplies cells to be sorted;

b. chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment;

c. sensing a property of said cells;

d. discriminating between cells having a desired sex characteristic; and

e. collecting cells having the desired sex characteristic.

129. A method of producing a mammal of a desired sex comprising the step of producing said mammal using the processes according to any of claims 18, 19, 20, 21, 22, or 23.

130. A method of producing a mammal of a desired sex as described in claim 124 and further comprising the step of sorting said cells at a high speed.

131. A method of sorting cells comprising the steps of :

a. establishing a cell source which supplies cells to be sorted;

b. chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment;

c. sensing a property of said cells;

d. discriminating between cells having a desired characteristic; and

e. collecting cells having the desired characteristic.

132. A method of sorting cells as described in claim 131 and further comprising the step of minimizing the chemical changes said cells are subjected to as a result of being subjected to said sheath fluid.

133. A method of sorting cells as described in claim 132 wherein said step of establishing a cell source comprises the step of establishing a source of non-repairing cells.

134. A method of sorting cells as described in claim 132 wherein said step of establishing a cell source comprises the step of establishing a source of sperm cells.

135. A method of sorting cells as described in claim 132 wherein said step of establishing a cell source comprises the step of establishing a source of bovine sperm cells.

136. A method of sorting cells as described in claim 132 wherein said step of establishing a cell source comprises the step of establishing a source of equine sperm cells.

137. A method of sorting cells as described in claim 134 and further comprising the step of inseminating a mammal using a low dose of said sperm cells.

138. A method of sorting cells as described in claim 135 wherein said step of chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is

coordinated with both a pre-sort and a post-sort cell fluid environment comprises the step of establishing a sheath fluid which contains about 2.9% sodium citrate.

139. A method of sorting cells as described in claim 136 wherein said step of chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment comprises the step of establishing a sheath fluid which contains a hepes buffered medium.

140. A method of sorting cells as described in claim 131, 132, or 134 and further comprising the step of sorting said cells at a high speed.

141. A method of sorting cells as described in claim 140 wherein said step of sorting said cells at a high speed comprises the step of subjecting said cells to a pressure of at least about fifty pounds per square inch.

142. A method of sorting cells as described in claim 134 wherein said step of collecting cells having the desired characteristic comprises the step of cushioning said cells from impact with a collection container.

143. A method of sorting cells as described in claim 1142 wherein said step of cushioning said cells from impact with a collection container comprises the step of providing a wide opening to said container.

144. A method of sorting cells comprising the steps of :

a. establishing a cell source which supplies cells to be sorted;
b. establishing a sheath fluid to create a sheath fluid environment for said cells;
c. sensing a property of said cells;
d. discriminating between cells having a desired sex characteristic;
e. collecting cells having the desired sex characteristic in a collector fluid; and

f. chemically coordinating said collector fluid to create an ending collector fluid environment for said cells which is coordinated with a pre-sort fluid environment.

145. A method of sorting cells as described in claim 144 wherein said step of establishing a cell source comprises the step of providing an initial nutrient for said cells and further comprising the step of providing a collection fluid nutrient for said cells and wherein said step of collecting cells having the desired characteristic in a collector fluid comprises the step of balancing said initial nutrient and said collection fluid nutrient after the completion of said step of collecting said cells.

146. A method of sorting cells as described in claim 145 wherein said step of collecting cells having the desired characteristic in a collector fluid comprises the step of establishing a citrate collection fluid containing about six percent egg yolk prior to commencing said step of collecting.

147. A method of sorting cells as described in claim 146 wherein said step of establishing a cell source comprises the step of establishing a source of bovine sperm cells.

148. A method of sorting cells as described in claim 144 and further comprising the step of inseminating a mammal using a low dose of said sperm cells.

149. A method of sorting cells comprising the steps of :

a. establishing a cell source which supplies cells to be sorted;

b. establishing a sheath fluid to create a sheath fluid environment for said cells;

c. sensing a property of said cells;

d. discriminating between cells having a desired sex characteristic;

e. collecting cells having the desired sex characteristic comprising the step of cushioning said cells from impact with a collection container.

150. A method of sorting cells as described in claim 149 wherein said step of cushioning said cells from impact with a collection container comprises the step of providing a wide opening to said container.

151. A method of sorting cells as described in claim 150 wherein said step of establishing a cell source comprises the step of establishing a source of mechanically delicate cells.

152. A method of sorting cells as described in claim 150 wherein said step of establishing a cell source comprises the step of establishing a source of sperm cells.

153. A method of sorting cells as described in claim 151 and further comprising the step of sorting said cells at a high speed.

154. A method of sorting cells as described in claim 153 wherein said step of sorting said cells at a high speed comprises the step of subjecting said cells to a pressure of at least about fifty pounds per square inch.

155. A method of producing a sexed sperm specimen comprising the step of creating said specimen using the processes according to any of claims 131, 134, 137, 140, 149, 144, or 147.

156. A method of producing a sexed sperm specimen as described in claim 155 and further comprising the step of sorting said cells at a high speed.

157. A method of producing a sexed sperm specimen as described in claim 155 and further comprising the step of providing said sexed sperm specimen for inseminating a mammal using a low dose of said sperm cells.

158. A method of producing a sexed sperm specimen as described in claim 156 and further comprising the step of providing said sexed sperm specimen for inseminating a mammal using a low dose of said sperm cells.

159. A method of producing a mammal of a desired sex comprising the step of producing said mammal using the processes according to any of claims 131, 134, 137, 140, 149, 144, or 147.

160. A method of producing a mammal as described in claim 159 and further comprising the step of sorting said cells at a high speed.

161. A method of producing a sexed sperm specimen as described in claim 159 and further comprising the step of inseminating a mammal using a low dose of said sperm cells.

162. A method of producing a sexed sperm specimen as described in claim 160 and further comprising the step of inseminating a mammal using a low dose of said sperm cells.

163. A method of producing multiple, sexed embryos from a female mammal comprising:

a. creating superovulation in said mammal to create at least two eggs comprising the step of using an ovulatory pharmaceutical to cause multiple eggs to be produced;

b. determining a sex of a sperm cell of a male mammal;

c. sorting according to said sex of said sperm cells;

d. inserting at least a portion of said sorted sperm cells into a uterus of said female mammal after an onset of estrus; and

e. fertilizing a plurality of said eggs in said uterus to produce multiple, sexed embryos.

164. A method of producing multiple, sexed embryos according to claim 163 wherein said creating superovulation is encouraged during the estrus cycle.

165. A method of producing multiple, sexed embryos according to claim 164 wherein said step of using a an ovulatory pharmaceutical comprises the step of injecting said ovulatory pharmaceutical in half days increments between any of days 2 and 18 of the estrus cycle.

166. A method of producing multiple, sexed embryos as described in claim 165 wherein said step for injecting said ovulatory pharmaceutical in half day increments comprises injecting at least seven injections and further comprising the step of incorporating an estrus manipulation system at least on about the sixth and seventh injections.

167. A method of producing multiple, sexed embryos as described in claim 166 wherein inserting at least a portion of said sorted sperm cells into said uterus comprises inserting said sperm cells into both uterine horns of said uterus.

168. A method of producing multiple, sexed embryos as described in claim 167 wherein inserting into both uterine horns comprises inserting said sperm cells approximately between 20 to 24 hours inclusive after said onset of said estrus.

169. A method of producing multiple, sexed embryos as described in claim 163 wherein said step of using an ovulatory pharmaceutical to cause multiple eggs to be produced comprises the step of injecting a dosage of follicle stimulating hormone a plurality of times a day.

170. A method of producing multiple, sexed embryos as described in claim 169 wherein said step of creating superovulation in said mammal to create at least two eggs further comprises the step of incorporating an estrus manipulation system comprising the step of supplementing said dosage of follicle stimulant hormone with prostaglandin F-2-alpha.

171. A method of producing multiple, sexed embryos as described in claim 170 wherein injecting said dosage of follicle stimulating hormone a plurality of times a day comprises injecting said follicle stimulating hormone in approximately half day increments at a dosage level of 6, 6, 4, 4, 2, 2, 2, and 2 mg between days 9 and 12 inclusive of the estrus cycle and wherein supplementing said dosage of follicle stimulating hormone with prostaglandin F-2-alpha comprises the step of injecting 25 and 12.5 mg of prostaglandin

F-2-alpha on the sixth and seventh dosages, respectively, of said follicle stimulating hormone.

172. A method of producing multiple, sexed embryos as described in claim 163 and further comprising the steps of:

a. staining sperm cells of a male mammal;

b. sorting according to said sex of said sperm cells through the use of high speed flow cytometry; and

c. concentrating said sorted sperm cells.

173. A method of producing multiple, sexed embryos as described in claim 163 wherein inserting at least a portion of said sorted sperm cells comprises using a low dose of said sperm cells.

174. A method of producing multiple, sexed embryos as described in claim 172 wherein inserting at least a portion of said sorted sperm cells comprises using a low dose of said sperm cells.

175. A method of producing a mammal of a desired sex comprising the step of producing said mammal using the processes as described in claims 163.

176. A method of producing a mammal of a desired sex comprising producing a sexed sperm cells mammal using the processes of claim 175 and further comprising sorting said cells at a high speed.

177. A method of producing mammal of a desired sex as described in claim 175 further comprising inseminating said female mammal using a low dose of said sperm cells.

178. A method of producing a mammal of a desired sex as described in claim 175 further comprising chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid

environment comprising establishing a sheath fluid which contains about 2.9% sodium citrate.

179. A method of producing a mammal of a desired sex as described in claim 178 wherein chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment comprises establishing a sheath fluid which contains a hepes buffered medium.

180. A method of producing a mammal of a desired sex as described in claim 179 further comprising collecting said sperm cells of said sex and cushioning said cells from impact with a collection container.

181. A method of sorting cells comprising:

a. establishing a cell source which supplies cells to be sorted;

b. chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort cell fluid environment;

c. sensing a property of said cells;

d. discriminating between cells having a desired sex characteristic; and

e. collecting cells having the desired sex characteristic.

182. A method of sorting cells as described in claim 181 and further comprising sorting said cells at a high speed.

VII. ABSTRACT

Artificial Insemination is achieved for sexed mammalian offspring in a commercially practical manner and with dosages of insemination sperm which were not previously thought to be practical for commercial implementation. Improved insemination systems particularly adapted to use for sex-selected sperm sorting include systems which achieve superovulation and then multiple embryo production with sexed embryos. These systems combine with other techniques, including techniques for enhanced sheath fluid (3) and other strategies which minimize stress on the sperm cells (18), and, potentially, a 2.9 percent sodium citrate sheath solution for bovine species and a hepes bovine gamete media for equine species. Improved collection systems (14) and techniques for the process are described so that commercial application of sperm samples as well as the resulting animals may now be achieved in the field.

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(57) Abstract

Artificial Insemination is achieved for sexed mammalian offspring in a commercially practical manner and with dosages of insemination sperm which were not previously thought to be practical for commercial implementation. Improved insemination systems particularly adapted to use for sex-selected sperm sorting include systems which achieve superovulation and then multiple embryo production with sexed embryos. These systems combine with other techniques, including techniques for enhanced sheath fluid (3) and other strategies which minimize stress on the sperm cells (18), and potentially, a 2.9 percent sodium citrate sheath solution for bovine species and a hepes bovine gamete media for equine species. Improved collection systems (14) and techniques for the process are describeb so that commercial application of sperm samples as well as the resulting animals may now be achieved in the field.